ASSEMBLY OF THE MITOCHONDRIAL MEMBRANE SYSTEM

Structure and location of the mitochondrial glutamic tRNA gene in *Saccharomyces cerevisiae*

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1. Introduction

Most of the mitochondrial tRNA genes of *Saccharomyces cerevisiae* have been mapped in the quadrant between the *cap* and *par* resistance loci [1–3]. The serine and glutamic tRNA genes, however, occur in a completely different region of the genome. The serine tRNA has been sequenced [4] and placed at 83 map units [5]. The glutamic tRNA has been mapped in the general vicinity of the cytochrome *b* gene, although it has not been precisely localized [1–3].

Here we report the sequence of the glutamic tRNA gene. The gene was sequenced in a segment of mitochondrial DNA (mtDNA) retained in the ρ^- clone DS400/A12. Based on the restriction map of the DS400/A12 genome, the gene has been placed at 69.9 map units between the *oli2* resistance locus and the cytochrome *b* gene.

2. Materials and methods

The DS400/A12 clone used in this study was isolated from the respiratory competent haploid *S. cerevisiae* D273-10B/A21 (α, met, ρ^+, $E^R_{624}O^R_{625}P^R_{626}$) by mutagenesis with ethidium bromide [6]. This clone retained all the *cob1* and *cob2* markers for cytochrome *b* but was deleted with respect to the flanking antibiotic resistance loci, *oli1* and *oli2*.

Yeast mitochondria were purified from protoplasts by the method in [7]. The mitochondria were lysed in buffered 2% Sarkosyl, phenol extracted and the DNA purified on CsCl–ethidium bromide gradients [8].

Restriction endonucleases were obtained from New England Biolabs, MA or were prepared as in [9]. Restriction fragments were treated with bacterial alkaline phosphatase (Worthington Biochem., NY) and labeled at the 5′-ends with bacteriophage T4 polynucleotide kinase (Bethesda Res. Labs, MD) and [γ-^{32}P]ATP (2000–3000 Ci/mmol, New England Nuclear Corp., MA) [10]. The labeled fragments were either strand separated on 6% polyacrylamide gels or cleaved with a second restriction enzyme prior to separation of the double-stranded fragments on polyacrylamide. The bands of interest were extracted from the gels and sequenced according to [10].

3. Results and discussion

DS400/A12 has a mitochondrial genome consisting of a circular DNA with a repeat unit length of 7.6 kbp [6]. The unique sequence retained in this clone has been established to extend from 69.3–80.2 units of the wild-type map [6]. This region of mtDNA contains all the cytochrome *b* markers but falls short of the flanking antibiotic resistance loci *oli1* and *oli2*.

We have reported the complete restriction map of the DS400/A12 mtDNA segment and the sequence from 71.4–80.2 units [6]. The sequence includes the entire structural gene of cytochrome *b* which was shown to be fragmented into 3 separate exons [6]. The region of DS400/A12 from 69.3–71.4 units proved to be A + T rich and was difficult to sequence due to the relatively few restriction sites available for labeling (fig.1). It was possible, however, to obtain the sequence near a *Taq* I/*Mbo* II/*Hin*fI/*Hph* I site cluster at 69.8 units. The DS400/A12 mtDNA was

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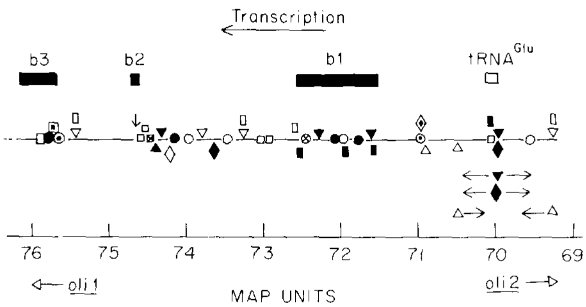

Fig.1. Restriction map of and location of the cytochrome *b* and tRNA^{Glu} genes in the mtDNA segment of DS400/A12. The restriction map of the DS400/A12 genome is shown from 69.3–76 map units. The following symbols are used to denote the restriction sites: *Hpa* II (△); *Hae* III (▯); *Hph* I (▮); *Alu* I (●); *Hin*fI (▲); *Mbo* I (○); *Mbo* II (□); *Taq* I (◆); *Hinc* II (⊙); *Eco* RI (⊗); *Eco* RII (↓); *Bcl* I (▣); *Bgl* II (⊡); *Hind* III (◇); *Hha* I (◈). The positions of the 3 cytochrome *b* exons (b1, b2, b3) are indicated by the solid bars and of the tRNA^{Glu} by the open bar. The arrows show the *Hin*fI and *Taq* I sites used to sequence the glutamic tRNA gene. The *oli1* and *oli2* markers are provided for the orientation of the mtDNA segment.

digested with *Taq* I. Following 5′-end labeling of the *Taq* I sites, the DNA was digested with *Hpa* II yielding two fragments of 440 and 400 bp. These were separated on 6% polyacrylamide and sequenced from the *Taq* I sites. Alternatively, the DS400/A12 DNA was digested with *Hin*fI. The two *Hin*fI fragments adjoining the site cluster at 69.9 units were isolated preparatively, digested with *Hpa* II and 5′-end labeled. The small *Hin*fI–*Hpa* II fragments were separated into single strands and used for sequencing (fig.1).

The sequence starting from either the *Hin*fI or *Taq* I sites at 69.9 units indicated that the site cluster occurs inside of a 75 nucleotide long sequence with a moderate G + C content. This short sequence is flanked by semi-repetitive A + T-rich sequences (fig.2). Part of the sequence (nucleotides +1 to +72) can be folded into a cloverleaf structure (fig.3). The anticodon in the derived secondary structure is 3′-CUU-5′ indicating that it is most likely to be the gene for the glutamic tRNA. The tRNA has the following standard features: a 7 bp acceptor stem; 5 bp in the TψC stem and 7 bases in the corresponding loop; 4 bases in the variable loop and 5 bp in the anticodon stem. The anticodon is bracketed by a pyrimidine (cytidine) on the 5′- and a purine (adenine) on the 3′-side. The D stem has some less common features being unusually large with 10 bases and only 3 base pairs in the stem.

The tRNA gene is located at 69.9 units, ~1160 nucleotides upstream of the initiation codon of the first cytochrome *b* exon. This localizes the tRNA between the cytochrome *b* gene and the *oli2* resistance

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                                                                                                   -315
                                                                                              5'-CCGGTCCCCGAAAGG
                                                                                                 HpaII
-300  GGAATAAGATAAATATATAAATTATATTAATAAATATAATTTAATGATTA TAATTAATATATATATGTATATATATATATATATATTAAATATATTTAAA

-200  TAGTTTCCGAAGGAGTGATAGATCCCTTTGGGGGGACCGAACCCCGAAAA GAATTTTATTTTATTTTTATTTTATTTAATTTAATTTTAATTAGGATAAT
                          MboI

-100  AAATAGTAATAATAAACTTAATAATAATAATAATAATTTTATTTTTATAA TTTATTAATAATAATAATAATTATATATATATATATTATTAATAAATATA

                                    MboII                   TaqI
  +1  GACCTTATCGTCTAATGGTTACGACATCACCTCTTCATGTTGATAATATC GGTTCGATTCCGATTAAGGTTATTCATAATAATAAATATTTGTAAAAAAG
                                HphI                        HinfI

+101  TATATATAATTAAACATATTCTTTATATTAATTAATAATTATTAATAATA TACATTTTATATAATACAATTATATATATATATATATTTTTTTTTAATAC

+201  AAATAATATATTCATAATAATAAATACCGATTGTTATTATACTATAATAA AATATATAATATATTTTTCATTATAATATTTTTAAATAAATATTATAATA

+301  AATTATATAAATAATATTTATGTATAATAATAATAATAATAATTGTTATT AATTAATTCTATAATTATTATATATTTAATTTTTTTTTTTTAATATAATAT

+401  ATAATAATATAATTTATTTTATTTTTTTTTTTATAGTTCCGG-3'
                                          HpaII
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Fig.2. Nucleotide sequence of the non-transcribed DNA strand in the region of the *Taq* I/*Mbo* II/*Hph* I/*Hin*fI site cluster. The sites have been marked in the sequence.

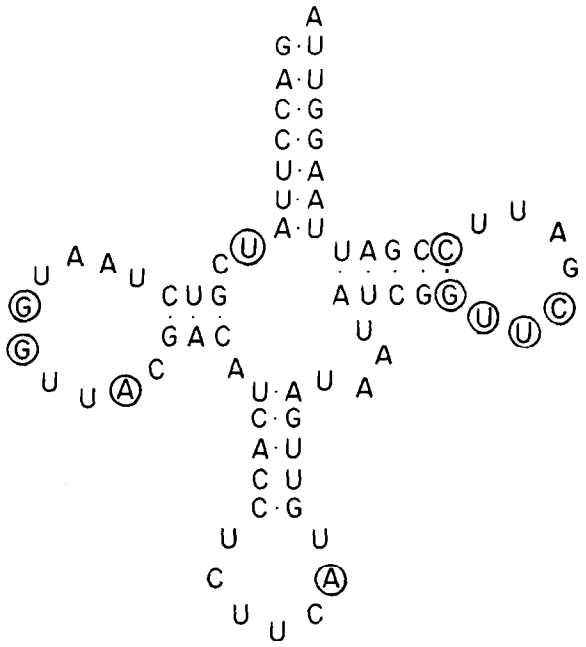

Fig.3. Proposed primary and secondary structure of yeast mitochondrial glutamic tRNA. The positions of the expected invariant bases are circled. All the thymidines have been replaced by uridine. Since the structure is derived from the DNA sequence, we do not know the location of possible modified bases.

locus. The glutamic tRNA is transcribed from the same DNA strand as cytochrome *b* and most other yeast mitochondrial gene sequences to date [11–13].

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